

REMARKS**I. Introduction**

In response to the Office Action dated July 14, 2005, claim 39 has been cancelled, and claims 17, 18, 37, 38 and 40 have been amended. Claims 17-35, 37, 38 and 40-44 remain in the application. Reconsideration of the application, as amended, is requested.

II. Claim Amendments

Applicants' attorney has made amendments to the claims as indicated above. These amendments were made solely for the purpose of clarifying the language of the claims, and do not introduce new matter or raise new issues. Support for these amendments can be found in the application as originally filed. Entry of these amendments is respectfully requested.

Claim 17 has been amended to delete the term "essentially" from "consisting essentially of", and to clarify "local direct administration". Support for these modes of administration can be found in the specification, e.g., at page 18, lines 1-4, and at page 25, lines 1-14.

Claim 18 has been amended to replace "ex vivo" with "in vivo", as supported by the specification at, e.g., page 12, line 31, page 13, line 4, and pages 56-63.

Claim 37 has been amended to delete the term "essentially" from "consisting essentially of", to be consistent with the amendment to claim 17.

Claim 38 has been amended to clarify that the DNA is delivered by local direct administration of a lipid-DNA complex comprising the replacement DNA fragment, as supported by the specification at, e.g., page 18, line 4, and page 25, lines 11-13.

Claim 40 has been amended to recite that the genetic defect is associated with cystic fibrosis, as supported by the specification at, e.g., page 7, line 26, and page 25, line 21.

III. Examiner Interview Summary

Record is made of a telephone interview on August 15, 2005, between Applicants' attorney Karen Canady, and Examiners Katcheves and Ketter in connection with the present patent application. Discussion during this interview centered on the *in vivo* data presented in the Declaration Under 37 CFR §1.132 by Dr. Dieter C. Gruenert, the data and arguments in support of

ex vivo methods, and potential claim amendments that could overcome outstanding rejections. The helpfulness of the Examiners in clarifying the remaining issues is appreciated. Applicants present the amendments and arguments herein in a good faith effort to advance prosecution and place the application in condition for allowance. Should the Examiner disagree, the courtesy of a telephone call to clarify any remaining issues would be most appreciated.

IV. Non-Art Rejections

At page 2 of the Office Action, claims 17-35 and 38-44 were rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for a method for replacing a target fragment of a cell *in vitro*, was not regarded as providing enablement for a method of replacing a target fragment *in vivo* or *ex vivo*, wherein the cells are intended for gene therapy use. Although the rejection is applied to all of claims 17-35 and 38-44 as a group, Applicants' response is presented separately for claims of varying scope. Throughout these arguments, reference is made to exhibits listed in the Table of Evidence submitted with the Amendment dated May 26, 2005, and a copy of this Table is provided again for reference.

A. Independent Claim 17

Claim 17 is directed to a method for replacing a target fragment of a gene in a cell. As discussed in the specification, this method is useful for many purposes, including constructing genetically modified cell lines that can be used, for example, to test drugs directed at a disease related to the genetic modification (see, e.g., pages 56-58). The method is also useful for creating transgenic animals (see, e.g., pages 58-63), as well as for modifying a gene in a cell to achieve a therapeutic benefit (pages 13-58). Numerous examples of this method of target replacement are provided in the specification as well as in the various exhibits presented during prosecution, using microinjection, electroporation and lipid-DNA complexes to deliver the replacement DNA fragment recited in claim 17 to the cell.

It is not disputed that the specification is enabling for a method for replacing a target fragment of a cell *in vitro* (see Office Action at page 2). The concern is whether the enablement is commensurate with the scope of the claims. It is noted at page 3 of the Office Action that the claims are very broad and encompass replacing a target fragment in a cell wherein the cell is *ex vivo*.

and wherein the cell is *in vivo*. An additional concern is raised about a potentially broad interpretation of the limitation "wherein the DNA is delivered by local direct administration".

In response, Applicants note the record shows that the replacement of a target fragment in a cell has been demonstrated in accordance with the method of claim 17 to make corrections in the cystic fibrosis gene, the β -globin gene associated with sickle cell anemia, and the *mdx* dystrophin gene associated with muscular dystrophy (see Table of Evidence). Applicants further note that these successful replacements have been performed in cell lines, primary airway epithelial cells, human umbilical cord blood stem/progenitor cells, embryonic stem cells, *in vivo* nasal mucosal cells, and *in vivo* intratracheal cells of the lung, and that these were achieved using microinjection, electroporation, and lipid-DNA complexes. Accordingly, the data supporting enablement of claim 17 encompasses a broad range of genetic defects, a broad range of cell types, and includes *in vitro*, *in vivo* and *ex vivo* environments. In addition, claim 17 has been amended to clarify the modes of administration as those which have been supported by the data and taught in the specification. Moreover, Applicants note that claim 17 is directed to a method of replacing a target fragment of a gene in a cell, a useful method in and of itself, regardless of therapeutic benefit, although the latter has been demonstrated as well.

B. Dependent Claim 18

Claim 18 depends from claim 17 and adds the limitation, "wherein the cell is *in vitro*". The enablement of the claimed method for *in vitro* cells is not disputed.

C. Dependent Claim 19

Claim 19 depends from claim 17 and adds the limitation, "wherein the cell is *in vivo*". The enablement of the claimed method for *in vivo* cells is supported by the Goncz et al. 2001 *Gene Therapy* 8:961-965 article submitted as Exhibit C on December 3, 2001, by the Kapsa et al. 2001 *Human Gene Therapy* 12:629-642 article submitted as Exhibit F on December 3, 2001, and by the data presented in the Declaration of Dr. Dieter Gruenert, also of record, at item 6, pages 3-4. Goncz et al. 2001 (Exhibit C) demonstrates successful *in vivo* fragment replacement and stable expression of altered DNA and mRNA in an animal model of cystic fibrosis wherein the replacement fragment was

delivered using three different agents: AVE, lipofectamine and DDAB. Although the Examiner alleges (page 11 of the Office Action) that none of the three agents used in this study is taught in Applicants' specification, this assertion is in error. Applicants' specification teaches numerous lipid-based delivery vehicles, including explicit mention of lipofectamine at page 42, line 11 (discussing the data presented in Figure 13). Kapsa et al. (Exhibit F) demonstrates successful *in vivo* replacement and correction of *mdx* dystrophin mutation in immune competent mice that was shown to last at least 3 weeks *in vivo*. Again, these data were dismissed as allegedly relying on delivery vehicles not taught in the specification because Kapsa et al. used lipofectamine and lipofectin. This statement is likewise in error. The specification explicitly mentions lipofectamine at page 42, line 11, and lipofectin at page 73, line 12. Accordingly, there is ample evidence that the claimed method, as taught in the application as filed, of replacing a target fragment of a gene in a cell *in vivo* is enabled.

D. Dependent Claims 20-35

Claims 20-35 recite more specific limitations that are clearly supported by the application as originally filed and enabling support for these limitations has not been questioned by the Examiner. Accordingly, Applicants maintain that dependent claims 20-35 are enabled by the specification.

E. Independent Claim 37

Independent claim 37 is directed to a composition comprising a replacement DNA fragment and a delivery vehicle suitable for delivery of the replacement DNA fragment into a cell, wherein the delivery vehicle comprises a lipid, a dendrimer or polylysine. Although claim 37 has been included with the remaining claims in the enablement rejections, Applicants find no basis in the arguments presented in the Office Action to support an assertion that the specification as filed fails to teach one of ordinary skill in the art, as of the application filing date, how to make and use the claimed composition. Both the working examples in the specification and the subsequent data summarized in the accompanying Table of Evidence demonstrate the ability of such compositions, using the claimed delivery vehicles, to effect replacement of a DNA fragment in a gene of a cell.

F. Dependent Claim 38

Claim 38 recites a method of gene therapy comprising contacting a cell with the composition of claim 37. Although the claimed method has been established to successfully result in replacement of a target fragment in a gene of a cell in a variety of disease models and using a variety of delivery methods, this claim has been amended so that its scope more closely tracks the data that have successfully demonstrated efficacy *in vivo*, and in particular with regard to the lipid-based delivery methods. The ability of the claimed method to achieve a therapeutically relevant benefit has been demonstrated by the data presented in the Declaration under 37 CFR §1.132 by Dr. Dieter Gruenert. Although this Declaration was acknowledged at pages 14-15 of the Office Action, the *in vivo* evidence of successful therapeutic application of the invention described at paragraph 6 of this Declaration appears to have been overlooked (again).

The data presented in the Declaration (see item 6) show short circuit current measurements that were made of nasal mucosa from ΔF508/ΔF508 mice (an animal model for cystic fibrosis) transfected with 786-bp fragments of mouse genomic (wild type) cystic fibrosis transmembrane conductance regulator (CFTR). Animals were transfected with lipofectamine DNA complexes that were added to the nose in 2-5 μ l aliquots. Analysis of the ion transport properties of the transfected ΔF508/ΔF508 mice indicated a mean increase in Cl⁻ ion transport greater than that observed with the lipid controls in 2 different experiments. These studies indicate that delivery of a wild-type mCFTR fragment into the nasal mucosa of ΔF508 CF mice will change the cAMP-dependent ion transport properties of the mice, in that they now secrete Cl⁻ in response to cAMP stimulation. Success with 2 different experiments is remarkable, given the limited availability of the ΔF508 animals and the variability of the parameters of the system (e.g., animal age, size, sex, feeding time before the experiment). In the 2 experiments where a significant increase in the cAMP stimulated I_{sc} was observed, the level of cAMP-dependent Cl⁻ transport was within a range comparable to that observed in normal animals.

These data were discussed with the Examiner and her supervisor during the telephonic interview held on August 15, 2005 and discussed above. The Examiner raised a concern that these data were not convincing because success was demonstrated in only two of the four experiments. Applicants direct the Examiner's attention to the difference in values observed in Experiments 1 and 3 as compared to the control groups. It is not credible, from a scientific standpoint, to dismiss the

difference between 23.1 ± 5.0 (n=5) or 32.5 ± 10.0 (n=5), and control levels of 8 ± 4.8 (n=7) and 2.4 ± 0.5 (n=5). These results cannot be attributed to chance alone. That a therapeutic effect was not achieved in each experiment does not explain away these results -- a remarkable achievement in gene therapy and homologous replacement! Certainly one would not deny that some antibody-based therapeutics offer a therapeutic benefit to some cancer patients even though not all patients respond equally to the same treatment strategy. Concerns about the ability to achieve success in all subjects in all experiments is not within the purview of the Patent & Trademark Office, but rather is a task assigned to the Food and Drug Administration ("FDA approval, however, is not a prerequisite for finding a compound useful within the meaning of the patent laws." *In re Brana*, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995) (citing *Scott v. Finney*, 34 F.3d 1058, 1063, 32 USPQ2d 1115, 1120 (Fed. Cir. 1994)). Applicants have shown that a substantial and statistically significant functional correction has been obtained with the claimed method. This is sufficient to demonstrate that the methods taught and claimed in the application are enabled.

These data, particularly when taken together with other *in vivo* data (Kapsa et al. and Goncz et al. discussed above) showing successful correction and expression following intratracheal and intramuscular administration, establish that one of skill in the art can practice the claimed method with a reasonable expectation of success using the teachings of the specification and the state of the art at the time the application was filed.

G. Dependent Claim 40

This claim, which depends from claim 38, recites limitations that track the data even more closely, by requiring the contacting occur *in vivo* and wherein the genetic defect is associated with cystic fibrosis. Accordingly, Applicants maintain that the full scope of claim 40 is clearly enabled.

H. Dependent Claims 41-44

Claims 41-44 recite more specific limitations that are clearly supported by the application as originally filed and enabling support for these limitations has not been questioned by the Examiner. Accordingly, Applicants maintain that dependent claims 41-44 are enabled by the specification.

I. Response to Arguments

At page 3 of the Office Action, a concern is raised that "local direct administration" could possibly encompass use of viral vectors. Applicants respectfully note that the claims do not encompass delivery using foreign vector material. The fragment to be delivered to the cell is limited to the correcting sequence (at least one replacement exon) flanked by noncoding sequence and a feature of the invention is that it does not require the use of vectors commonly used in other methods of genetic correction or gene therapy.

At page 4 of the Office Action, a concern is raised regarding problems with existing methods of gene therapy. This is precisely why we have a patent system: to encourage disclosure of new developments that overcome limitations of existing technology. By setting the bar for patentability even higher, or by assuming that limitations of the prior art apply to the new, claimed method, the disclosure of such innovations is discouraged. The Patent Office is respectfully urged to consider the advantages offered by the claimed method that overcome problems of other methods that require use of foreign vector material and foreign genes. This argument applies as well to the concern raised at page 5 of the Office Action, regarding problems with heterologous gene expression. One advantage of the claimed method is that it obviates the need for heterologous genes.

At page 5 of the Office Action, problems noted with *ex vivo* gene therapy methods and the ability of such modified cells to out-compete native cells overlooks the reality that sickle cells have 1/8 to 1/10 the half life of normal cells, putting the native cells at a competitive disadvantage. The concern is not applicable to *ex vivo* gene therapy that corrects a defect that makes the diseased cells unable to successfully compete in the first instance (e.g., sickle cell anemia and severe combined immunodeficiency). Although Applicants have deleted "*ex vivo*" from the claims, Applicants maintain that the claimed method does in fact work in an *ex vivo* context. Applicants' data in support of *ex vivo* gene therapy (Prokopishyn et al. manuscript, Exhibit A) was dismissed by the Examiner because the host animals were immune deficient mice. It appears the Patent Office requires that this experiment be repeated either using animal cells or human hosts, neither of which is practical and realistic at the present time. Applicants have presented data using an *arr-accepted* animal model for stem cell re-population. Although Applicants disagree that data from immune

competent (and, therefore, human or autologous) hosts are required to establish enablement, "ex vivo" has been deleted from the claims in the interest of advancing prosecution.

At page 6 of the Office Action, a concern is raised that "one would have to further determine how to ensure replacement of both copies" of the defective exon. This, in fact, has been done. The Prokopishyn manuscript (Exhibit A submitted on September 26, 2003; see Table of Evidence) demonstrates successful replacement at both alleles of the β -globin gene using a 559 bp fragment containing exons 1 and 2. Again, this is an argument that has been addressed repeatedly by Applicants, and yet continues unchanged in communications from the Patent Office.

The use of post-filing date references is relevant to determining enablement, so long as the references address the state of the art at the time of the filing and use the guidance taught in the specification (see *In re Hogan* 559 F.2d 595, 605, 194 USPQ 527, 537 (CCPA 1977). Moreover, at page 10 of the Office Action, the Ferber article (*Science* 294, 2001) was criticized as a post-filing reference. This article was cited as evidence of how those skilled in the art view the Applicants' invention. To suggest that it would only be useful had it been published before Applicants' invention became public misses the point and implies an absurd requirement for the consideration of such evidence. In addition, this article was noted by the Examiner as indicating only that one skilled in the art would recognize Applicants' method as "promising, not that it would be successful". This comment implies that being viewed favorably by the scientific community is insufficient; to be considered enabled the scientific community would have to be convinced of its success. This is not the standard; all that is required is that Applicants' show that it would have been more likely than not (preponderance of the evidence) that the teachings of the specification would give one skilled in the art a reasonable expectation of success.

As noted throughout the arguments above, much of the evidence submitted by Applicants in support of enablement was dismissed by the Examiner for use of delivery vehicles not taught in the specification. The Examiner is urged to note that these assertions are in error. Each and every source of data listed on the Table of Evidence submitted herewith involved the use of microinjection, electroporation, lipofectamine, liposomes, lipofectin and/or lipid-DNA complexes, all of which are taught in the specification.

V. Prior Art Rejections

At page 6 of the Office Action, claims 17-20, 26-39, 31 and 37-44 were rejected under 35 U.S.C. §103(a) as being unpatentable over Vega (*Human Genetics* 1991, 87:245-253). This rejection relied upon an interpretation of the claims as using open language in the recitation of "consisting essentially of". The deletion of "essentially" renders this rejection moot. Applicants note for the record that "consisting essentially of" was not intended to have the same meaning as "comprising", as discussed in the Amendments submitted on September 26, 2003, and May 26, 2005.

VI. Obviousness Double Patenting

At page 8 of the Office Action, claims 17, 20-26 and 28-36 were rejected as previously indicated under the doctrine of obviousness double patenting. Applicants' intent to file a terminal disclaimer upon indication of allowable subject matter was noted. Applicants await final resolution of allowable claim language prior to submission of a terminal disclaimer.

VII. Conclusion

In view of the above, it is submitted that this application is now in good order for allowance and such allowance is respectfully solicited. Should the Examiner believe minor matters still remain that can be resolved in a telephone interview, the Examiner is urged to call Applicants' undersigned attorney.

Respectfully submitted,

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Enclosure

KSC/bj: G&C 30448.97-US-D1

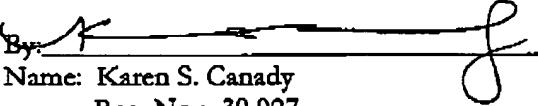
By: 
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Table of Evidence Supporting Enablement
U.S. Patent Application Number 09/392,862

<u>Data Source</u>	<u>Delivery Method</u>	<u>Delivery Vehicle</u>	<u>Fragment¹</u>	<u>Model System</u>	<u>Result</u>
Declaration of Dr. Gruenert (Item 6, p. 3-4)	In Vivo (nasal administration)	Lipofect-amine	786 bp containing wild type exon 10 of mCFTR	Animal model of cystic fibrosis: ΔF508	Successful in vivo gene therapy: functional correction of ion transport to normal ranges in immune competent mice
Prokopishyn et al., manuscript, "Targeted Genome Editing . . .", Exhibit A of 9/26/03	Ex Vivo (human umbilical cord blood stem/progenitor cells engrafted into mice)	Micro-injection	559 bp containing exons 1&2 of β ^s -globin gene	Sickle cell anemia: wild type β ^A -globin replaced w/ β ^s -globin mutation	Successful replacement and engraftment of corrected stem cells into immune deficient mice; replacement at both alleles; sufficient levels of gene conversion to support therapeutic benefit
Goncz et al. manuscript, "Modification of Genomic . . .", Exhibit B of 9/26/03	In Vitro	Micro-injection	559 bp containing exons 1&2 of β ^s -globin mutation	Sickle cell anemia: wild type β ^A -globin replaced w/ β ^s -globin mutation	Successful & stable in vitro replacement of genomic DNA and successful mRNA expression
Goncz et al. 2001, <i>Gene Therapy</i> 8:961-965, Exhibit C of 12/3/01	In Vivo (into lungs via intratracheal administration)	1. AVE 2. Lipofect-amine 3. DDAB	783 bp containing exon 10 of mCFTR w/ ΔF508	Animal model of cystic fibrosis: ΔF508	Successful in vivo fragment replacement and stable expression of altered DNA & mRNA
Kunzelmann et al. 1998, <i>Gene Therapy</i> 3:859-867, Exhibit D of 12/3/01	In Vitro (into cystic fibrosis epithelial cells)	1. Liposomes 2. Poly-amido-amine dendrimers	491 bp containing wild type exon 10 of CFTR	Cystic fibrosis: ΔF508	Successful replacement & correction of defect confirmed in genomic DNA and in mRNA; functional correction of ion transport confirmed by patch clamp
Goncz et al. 1998, <i>Human Molecular Genetics</i> 7:1913-1919, Exhibit E of 12/3/01	In Vitro (into primary human airway epithelial cells)	1. Liposomes 2. Poly-amido-amine dendrimers	488 nt containing exon 10 of CFTR w/ ΔF508	Cystic fibrosis: ΔF508	Successful fragment replacement & correction of defect confirmed in genomic DNA and in mRNA
Kapsa et al. 2001, <i>Human Gene Therapy</i> 12:629-642, Exhibit F of 12/3/01	In Vivo (intramuscular injection) and In Vitro (applied to cultured cells)	1. Lipofect-amine 2. Lipofectin	603 bp containing exon 23 of dys gene	Animal model of Duchenne muscular dystrophy: mdx	Successful in vivo and in vitro replacement and correction of mdx dystrophin mutation in immune competent mice; shown to last up to 28 d in culture and at least 3 wks in vivo.
Goncz et al. 2001, abstract, Technologies for <i>in situ</i> Repair . . ., Exhibit G of 12/3/01	In Vitro	Lipid-DNA complexes Micro-injection	Fragments containing β ^s -globin mutation	Sickle cell anemia: wild type β ^A -globin replaced w/ β ^s -globin mutation	Successful in vitro replacement at β-globin locus in hematopoietic cells; Replacement does not require transcription; Stable replacement lasts at least 5 wks

¹ Each fragment includes intronic sequence flanking the indicated exon(s).